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Review Hypothesis

Gene duplication as a means for altering H^+/ATP ratios during the evolution of F_oF_1 ATPases and synthases

Richard L. Cross and Lincoln Taiz*

Department of Biochemistry and Molecular Biology, SUNY Health Science Center, Syracuse, NY 13210 and **Biology Department, University of California, Santa Cruz, CA 95064, USA*

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In the evolution of the F_oF_1 family of proton-translocating membrane complexes, two reversals in function appear to have occurred, first changing it from an ATPase to an ATP synthase and then back again to an ATPase. Here we suggest that with each change in function, the ratio of protons transported per ATP hydrolyzed or synthesized (H^+/ATP) was altered in order for the complex to better adapt to its new role. We propose that this was accomplished by gene duplication with partial loss in the number of functional catalytic sites (to increase H^+/ATP) or functional proton channels (to decrease H^+/ATP). This method of changing the H^+/ATP ratio preserved overall structural features of the complex essential to energy coupling.

Evolution; H^+/ATP-ratios, Proton-translocating ATPase; ATP synthase; Gene duplication

1. INTRODUCTION

The F_oF_1 family of proton-translocating ATPases and ATP synthases includes two related groups: the F-type complexes found in eubacteria, mitochondria, and chloroplasts, and the V- or vacuolar-type complexes found in archaebacteria and eukaryotic vacuoles. The principal function of the ATPases is to couple the hydrolysis of ATP to the transport of protons up an electrochemical gradient, whereas the ATP synthases couple proton transport down a gradient to the synthesis of ATP[1]. During evolution, the predominant direction of proton flow through the complex appears to have reversed on at least two different occasions. Early life forms derived energy from fermentation, yielding organic acids as end-products. F_oF_1 is thought to have first evolved as an ATP-driven proton pump which used glycolytic ATP to maintain a near neutral internal pH and to drive proton/metabolite symports. The evolution of proton-pumping photosystems and electron-transport chains provided an alternative means of extruding protons. Indeed, energy derived from sunlight and the oxidation of nonfermentable substrates was so abundant, the capacity for pumping protons by these alternative systems may have quickly exceeded that necessary for maintaining pH and driving transport. This would have allowed the direction of proton flow through the ancestral F_oF_1 to reverse, thereby converting it from an ATPase to an ATP synthase [1]. The descendants of these photophosphorylation and oxidative phosphorylation systems are found today in chloroplasts, mitochondria, eubacteria, and archaebacteria.

At least one further reversal of function appears to have occurred. A progenitor of the current archaebacterial ATP synthase is believed to have given rise to eukaryotic vacuolar ATPase [2,3]. The sole function of these endomembrane ATPases is to couple ATP hydrolysis to the generation of an electrochemical gradient that lowers intravacuolar pH and drives secondary transport processes [4].

In this paper, we propose that with each switch in function, the ratio of protons transported per ATP hydrolyzed or synthesized (H^+/ATP) was altered to give an optimal value. We suggest that this altered coupling ratio was accomplished by gene duplication without any change in spatial relationships between subunits in the complex that would have interfered with energy coupling.

ATP synthases found in mitochondrial, chloroplast, and eubacterial membranes have been the most extensively studied [5]. They contain 8 or more different subunits, some of which are present in multiple copies.

Correspondence address: R.L. Cross, Department of Biochemistry and Molecular Biology, SUNY Health Science Center at Syracuse, Syracuse, NY 13210, USA

[1] Under the appropriate conditions, the reaction catalyzed by ATP synthases can readily be made to run in reverse. For this reason they are often referred to as ATPase. However, in this paper we are only concerned with their normal physiological function and use of the term ATPase will be restricted to complexes that function primarily as ATP-driven proton pumps.

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The complex has an extrinsic domain, F_1, that can be readily dissociated from the membrane. Solubilized F_1 retains the capacity for ATP hydrolysis and contains 3 copies of the catalytic subunit. A second major subunit is also present in 3 copies. This subunit lacks catalytic activity, but is paralogous to the catalytic subunit, i.e. derived from a gene duplication. Together these large subunits form a hexameric structure in which the catalytic and noncatalytic subunits alternate in positions around a puckered ring [6].

When F_1 is removed, the membrane retains a complex of hydrophobic subunits, F_o, capable of transporting protons. One component of this complex, the c-subunit, contains an essential carboxyl residue believed to participate in proton transport. The number of c-subunits in ATP synthases is not known with certainty, but most investigators in the field accept a value of 9–12. For the purposes of this discussion, we will assume the presence of 12 copies. We will also assume that in one complete catalytic turnover of the F_oF_1 complex, the number of protons transported will equal the number of c-subunits in F_o, whereas the number of ATP molecules formed or cleaved will equal the number of catalytic subunits in F_1.

2. PROPOSAL

It is proposed that the ancestral F_oF_1 complex which first appeared in anaerobic bacteria had 12 copies of the c-subunit and 6 copies of the catalytic subunit (**I**, fig.1). This structure would have a predicted stoichiometry of 12 protons pumped (12 H^+ channels) per 6 ATP hydrolyzed (6 catalytic sites) or 2.0 H^+/ATP.

Such a ratio might be expected to be well suited to a role for F_oF_1 in pumping protons out of the cell. However, when the direction of proton flow reversed at the time the complex evolved into an ATP synthase, this ratio may have been inadequate for maintaining a proper phosphorylation potential. This is readily illustrated by considering the thermodynamic relationship between the two coupled reactions: $\Delta G(\mathrm{ATP}) = -n\Delta\bar{\mu}$ (H), whereas ΔG(ATP) is the free energy change for ATP synthesis or hydrolysis, n is the H^+/ATP ratio, and $\delta\bar{\mu}(H^+)$ is the free energy change for proton movement down (for ATP synthesis) or up (for ATP hydrolysis) the electrochemical gradient. Thus, when the main function of the complex is to extrude protons against an electrochemical gradient, a low H^+/ATP ratio will allow a given ΔG(ATP) to pump protons against a large $\Delta\bar{\mu}(H^+)$. Conversely, when its main function is to synthesize ATP, a high H^+/ATP ratio will allow a given $\Delta\bar{\mu}(H^+)$ to drive ATP synthesis against a large ΔG(ATP).

Accordingly, we propose that as the ancestral F_oF_1 ATPase evolved into an ATP synthase, the H^+/ATP ratio was increased from 2.0 to 4.0 by a process that involved duplication of the gene for the catalytic subunit. Following gene duplication, mutations in one of the genes resulted in a loss of catalytic function, while the synthesis and assembly of the defective subunit continued. This resulted in the retention of the hexameric structure of F_1, but instead of 6 catalytic subunits, the hexamer now contained 3 'noncatalytic' (NC) plus 3 catalytic (CAT) subunits (**II**, fig.1). This method of altering the H^+/ATP ratio might be expected to be preferable to reducing F_1 from a hexamer to a trimer, since such a large decrease in size of F_1, relative to F_o, might be incompatible with the coupling mechanism.

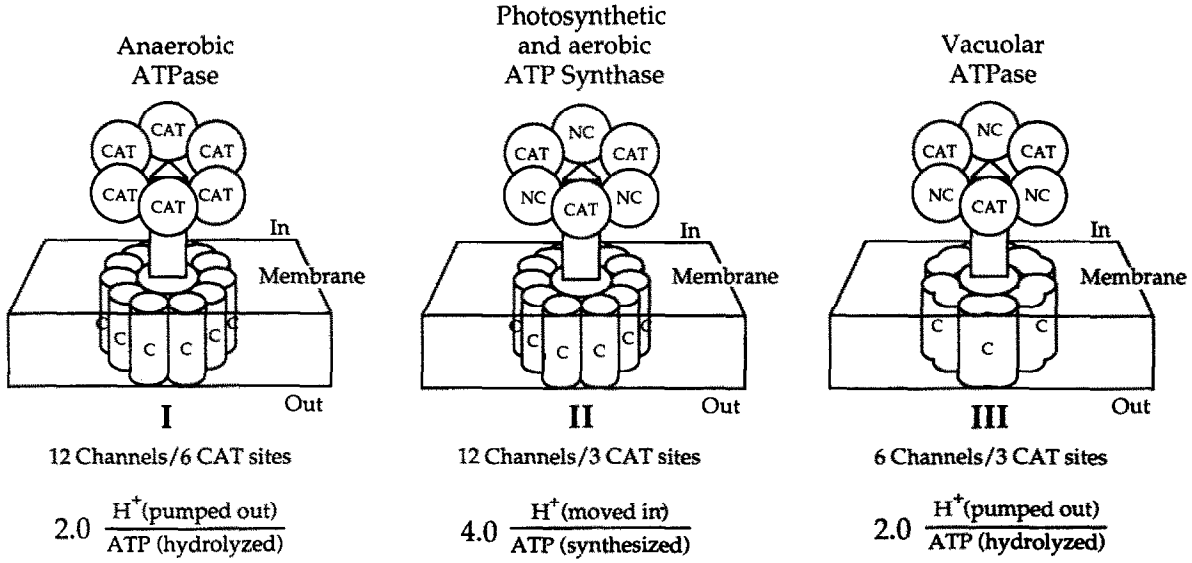

Fig.1. A scheme for the evolution of the F_oF_1 family of proton-translocating ATPases and ATP synthases. The first reversal in direction of proton flow through the complex occurred when an ancestral ATPase evolved into an ATP synthase (**I** → **II**). Accompanying this change in function, we propose that gene duplication of the catalytic subunit resulted in a loss of one-half of the catalytic sites and a doubling of the H^+/ATP ratio. It is further proposed that the second reversal in the direction of protein flow through the complex (**II** → **III**) was accompanied by gene duplication of the c-subunit resulting in a loss of one-half of the proton pores and a two-fold decrease in the H^+/ATP ratio. 'In' designates the side of the membrane that faces the cytosol in all cases except mitochondria where it designates the matrix side.

The second reversal in direction of proton flow occurred when the progenitor of archaebacterial ATP synthase gave rise to eukaryotic vacuolar ATPase. With this change in function, a low H^+/ATP ratio was again desirable. Simple deletion of the gene for the noncatalytic subunit with a return to a hexamer of catalytic subunits may not have been a viable option for accomplishing this. The catalytic subunit of archaebacterial ATP synthase appears to have aquired a large insert making it 25% larger than the corresponding subunit found in other synthases [3,7-9]. Furthermore, prior to the appearance of eukaryotes, there would have been ample time for residues at the contact surfaces between the catalytic and noncatalytic subunits to mutate in such a way as to favor an alternating arrangement of the subunits. Such changes may have led to an irreversible loss in ability of the catalytic subunit to assemble into a fully functional hexamer. Instead, we propose that the H^+/ATP ratio of vacuolar ATPase was decreased by a process involving duplication of the gene for the c-subunit followed by gene fusion. Structure **III** in fig.1 depicts 6 copies of the bulkier c-subunit lining the perimeter of the F_o complex. Subsequent mutations in the new gene included the loss of one of the two essential carboxyl residues involved in transport. According to this model, the number of c-subunits was reduced from 12 to 6 and the H^+/ATP ratio was reduced from 4.0 to 2.0[2].

3. SUPPORTIVE EVIDENCE

The assumption that the H^+/ATP ratio is equal to the ratio of c-subunits to catalytic subunits is supported by information available regarding ATP synthases (**II**, fig.1). There appear to be 9–12 copies of the c-subunit [10] vs 3 copies of the catalytic subunit [11–14], and the c-subunit contains a carboxyl residue that is essential for proton transport [15]. Furthermore, kinetic [16] and thermodynamic [17] data support a stoichiometry of at least 3 H^+ transported per ATP synthesized.

Our proposal that the first reversal in function of F_oF_1 (ATPase → Synthase) was accompanied by the loss of one-half of the catalytic sites is supported by the presence in ATP synthases of 3 catalytic and 3 noncatalytic subunits that arose by gene duplication prior to the divergence of eubacteria and archaebacteria [3]. The amino acid sequences of noncatalytic subunits show 25% identity to catalytic subunits but lack several highly conserved essential residues found in catalytic subunits [18].

Our proposal that the second reversal in function (Synthase → ATPase) was accompanied by a partial loss of functional proton channels is based on the finding that the vacuolar c-subunit (16 kDa) is approximately twice the size of c-subunits from other sources (9 kDa). Both halves of the vacuolar c-subunit show homology to each other and to the c-subunits of ATP synthases, although the essential carboxyl group is missing from the N-terminal half of the molecule [19]. Furthermore, vacuolar ATPases contain 3 copies each of subunits homologous to the catalytic and noncatalytic subunits of ATP synthases and approximately 6 copies of the c-subunit [20], while the measured H^+/ATP ratio is 2 [21,22].

[2] Alternatively, structures **I** and **II** may contain 9 copies of the c-subunit, in which case the number of c-subunits dropped from 9 to 6 and the H^+/ATP ratio dropped from 3 to 2 in going from structure **II** to **III**. The accomodation of more than one-half as many double-sized subunits (i.e. 6 in place of 9) could conceivably occur without disrupting the coupling process if the c-subunits are located at the perimeter of the F_o complex.

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